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Atty. Dkt. No. 073406-0701

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: PELLETIER et al.

Title: COMPOSITIONS AND METHODS INVOLVING AN ESSENTIAL STAPHYLOCOCCUS AUREUS GENE AND ITS ENCODED PROTEIN STAAU_R9

Appl. No.: 10/025,222

Filing Date: December 19, 2001

Examiner: Not Known

Art Unit: Not Known

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to: **Commissioner for Patents, Washington, D.C. 20231**, on the date below.

Deborah L. Wykes
(Printed Name)

Deborah L. Wykes
(Signature)

May 22, 2002
(Date of Deposit)

AMENDMENT

Commissioner for Patents
Box NON-FEE AMENDMENT
Washington, D.C. 20231

Sir:

This communication is responsive to the Office Action dated March 15, 2002, concerning the above-referenced patent application.

Please amend the application as follows:

In the Drawings:

Please substitute the attached 24 sheets (Figs. 1 through 11) of corrected formal drawings for the drawings originally filed with the application.

In the Specification:

Please amend the specification as follows:

Insert the Sequence Listing submitted herewith.

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Insert the substitute amended paragraphs provided in Appendix 1. Marked-up copies of the amended paragraphs are provided in Appendix 2.

REMARKS

The substitute amended paragraphs were amended to insert or correct reference to SEQ ID Nos., to correct a typographical error, and to remove an executable link. Thus, the amendments do not represent new matter.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

Date 22 May 2002

By _____

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Atty. Dkt. No. DOCKET_NO

Appendix 1 – Clean copies of substitute amended paragraphs

Replace the Paragraphs on p.15, line 25 to p.16, line 14.

Fig. 6 shows the tryptic peptide mass spectrum analysis of the PT72 protein interacting with 96 ORF 78. The gel slice containing PT72 contained one protein. The PT72 band was identified as an open reading frame, herein referred as STAAU_R9, found in Contig 286 of the University of Oklahoma genome sequencing project database (Web site with the remainder of the address being genome.ou.edu/staph.html) (SEQ ID NOS 29-36).

Fig. 7 shows the results of amino acid sequence analysis of STAAU_R9. A) Results of the STAAU_R9 Hidden Markov Model (HMM) searching analysis of the publically available Pfam database identifying two conserved Pfam motifs: Zf-CHC2(SEQ ID NO: 37) compared with STAAU_R9 (residues 3-100 of SEQ ID NO: 2) and Toprim(SEQ ID NO: 38) compared with STAAU_R9 (residues 260-339 of SEQ ID NO: 2). B) Results of the global optimal alignment of the amino acid sequences of different STAAU_R9-related sequences. STAAU_R9(SEQ ID NO: 2) is highly similar to *S. aureus* DNA primase (SEQ ID NO: 39) (92% identity to gi|2494147|sp|O05338|PRIM_STAAU DNA PRIMASE, DnaG). Note the discrepancies between the sequences of DNA primase from *S. aureus* as reported in Swissprot and as predicted from the University of Oklahoma *S. aureus* genome sequencing project database. STAAU_R9(SEQ ID NO: 2) is also moderately similar to a variety of bacterial DNA primase proteins including *B. stearothermophilus* DnaG (SEQ ID NO: 40) (34% identity to gi|9910841|sp|Q9X4D0|PRIM_BACST DNA PRIMASE) *B. subtilis* DnaG(SEQ ID NO: 41) (36% identity to gi|130904|sp|P05096|PRIM_BACSU DNA PRIMASE) and *E. coli* DnaG (SEQ ID NO: 22) (27% identity to gi|130908|sp|P02923|PRIM_ECOLI DNA PRIMASE).

Substitute amended paragraph for p.17, lines 22-26.

Fig. 11 shows the list of the oligonucleotide primers (SEQ ID NOS 8-21 and 7, respectively in order of appearance) used for amplification by PCR and cloning of

the *S. aureus* STAAU_R9-related sequences in vectors for the yeast two-hybrid analysis. A) Sequence of each primer with the restriction site used for cloning identified; B) pairs of primers used to clone the full-length STAAU_R9 and the thirteen STAAU_R9-related fragments.

Substitute amended paragraph for p.101, line 28 to p.102, line 23.

As shown in Fig. 7B, the result of the optimal global amino acid sequence aligment of STAAU_R9 with the described *S. aureus* DnaG (Swissprot No: O05338) reveals a 92% identify between the two polypeptides. The discrepancies between the sequences of DNA primase from *S. aureus* as reported in Swissprot and as reported in the University of Oklahoma *S. aureus* genome sequencing project database is noteworthy. The N-terminal sequence of STAAU_R9 (SEQ ID NO: 2) was predicted based on the presence of a fragment of 1171.623 in the mass spectrum (Fig. 6). This tryptic-digested fragment corresponds to the mass predicted from the sequence (SEQ ID NO: 31: IDQSIINEIK) extending from amino acid residue 5 to 14 of the deduced amino acid sequence of STAAU_R9. In addition, the 5' DNA sequence of STAAU_R9 on the genome of *S. aureus* strain RN4220 was confirmed by PCR and DNA sequence analyses with the following primer pair; (SEQ ID NO: 25) 5'-GCGCATCTGTAAAACCACG-3' AND (SEQ ID NO: 26) 5'-GCACGAATTCAAGAAGAATTG-3'. Fig. 7B also shows that STAAU_R9 is similar to several bacterial DNA primases including DnaG polypeptides of *B. stearothermophilus, B. subtilis* and *E. coli*, with identities of 34%, 36% and 27%, respectively.Fig. 7A shows the results of the STAAU_R9 Hidden Markov Model searching analysis of the publicly available Pfam database identifying two highly related Pfam motifs in the STAAU_R9 region spanning amino acid position 1 to 339. STAAU_R9 harbors a N-terminal zinc finger-binding domain that could be involved in template DNA recognition and a Toprim domain, located centrally, and which corresponds to a conserved catalytic domain in bacterial DnaG-type primases. The C-terminal region of STAAU_R9 is only weakly conserved amongst bacterial DNA primases as exemplified in the optimal global amino acid sequences alignment presented in Fig. 7B.

Atty. Dkt. No. DOCKET_NO

Appendix 2 – Marked-up copies of amended paragraphs

Marked up amended paragraphs at p.15, line 25 to p.16, line 14.

Fig. 6 shows the tryptic peptide mass spectrum analysis of the PT72 protein interacting with 96 ORF 78. The gel slice containing PT72 contained one protein. The PT72 band was identified as an open reading frame, herein referred as STAAU_R9, found in Contig 286 of the University of Oklahoma genome sequencing project database ~~(http://www.genome.ou.edu/staph.html)~~ (Web site with the remainder of the address being genome.ou.edu/staph.html) (SEQ ID NOS 29-36).

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